IN THE UNITED STATES PATENTS AND TRADE MARK OFFICE

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
Title: Method of Water Purification
Examiner Ivars C. Cintins

DECLARATION UNDER Rule 132

I, Marilyn Karaman of 105 Bandjalong Crescent, Aranda, ACT, Australia, declare that:

1. I am one of the inventors of the subject matter of US Patent Serial No. 09/646347 (hereinafter referred to as "the present application") filed on 18 March 1999.

2. My qualifications and technical experience are set out in my *Curriculum vitae*, a copy of which is attached as Annex A.

3. My *Curriculum vitae* demonstrates that I have substantial experience in the art of water purification and particularly the removal of the protozoa from water.

4. The invention of the present application results from a study of the flocculation process for a variety of water treatment chemicals and their interaction with environmental pathogens such as *Cryptosporidium parvuum* (hereinafter referred to as *Cryptosporidium*).

5. Looking at *Cryptosporidium* particularly, we tested a range of inorganic solids including fluorspar, geothite, rutile, pyrite and silica along with hydroxylated alumina (CryptoBlast™) to measure each of their ability to adsorb oocysts of *Cryptosporidium in vitro*. The results of this experiment are shown in Figure 1, attached herewith as Annex B.

6. With the exception of hydroxylated alumina, the results indicate that oocysts of *Cryptosporidium* readily pass through columns filled with each of the inorganic solids tested. The experiment showed that, of all the solids tested, the oocysts strongly adsorbed onto the hydroxylated alumina only.

7. The next question was whether the alumina must be hydroxylated to have its effect on adsorbing oocysts of protozoa or whether unhydroxylated alumina would have the same efficacy. In this regard, we carried out a series of experiments using 200μm unhydroxylated alumina particles.

The unhydroxylated alumina was prepared by heating hydroxylated alumina at 610^0C in the presence of air for two and a half hours such that the hydroxyl groups were removed.

8. Around 1 gram of 200μm unhydroxylated alumina was tested in a column with a dispersion of *Cryptosporidium* in water, the results of which are shown in Figure 2, attached herewith as Annex C.

9. We found that, upon addition of the *Cryptosporidium* containing water to the column, around 10% of the *Cryptosporidium* would be immediately released (permeate in Figure 2). Furthermore, further washing of the column with water removed the remaining *Cryptosporidium*. Thus, washing the column for the fifth time with water resulted in release of almost 75% of the *Cryptosporidium* from the column.

10. As indicated in Figure 2, we observed the complete opposite behaviour for hydroxylated alumina. In this case, the *Cryptosporidium* were irreversibly adsorbed on the hydroxylated alumina and no *Cryptosporidium* were released by washing the column with water.

11. I have undertaken a review of US Patent No. 6,054,059 by Latimer *et al* (hereinafter referred to as the "Latimer patent").

12. The Latimer patent describes a filtration material which includes mineral substrate coated with various metal oxides such as Al_2O_3, MgO or SiO_2. The "coating" of metal oxide is achieved by heating mixtures of the mineral substrate and the metal oxides at temperatures between 2000 and 2200 °F.

13. Under these temperature conditions, the metal oxides Al_2O_3 (and SiO_2) will lose their surface hydroxyl groups giving rise to the formation of unhydroxylated, hydrophobic coatings on the mineral substrate.

14. The Latimer patent would appear to rely upon electrical affinity between the metal oxides and the protozoa as discussed at column 8 line 64 to 67:

"In addition to providing the filtration material with a desired electrical affinity, the surface metal oxides serve as parting agents to prevent the prills from sticking together as the intense heat is applied during firing."

It is unclear from a reading of this document as to how the alumina in an unhydroxylated form can act in this manner to remove protozoa from water.

Further, the surface of the agglomerated fine minerals would not be completely coated with the unhydroxylated alumina. In this regard, I refer to column 9, lines 3-9 of the Latimer patent:

"During firing, some of the surface may be occupied by the surface metal oxide and other portions of the surface may be occupied by the mineral fines. If magnesium oxide is used, a higher percentage of magnesium oxide on the surface may be required compared to the amount of aluminium oxide on the surface"

15. The hydroxylated alumina of our invention does not rely upon electrostatic charges as suggested by the Latimer patent. Rather, the present invention relies on the strong, specific chemical interaction between hydroxylated alumina and carboxylic groups present on the surface of protozoa. Furthermore, our experimental work shows that the adsorption of protozoa on the hydroxylated alumina is independent of the charge of the alumina and adsorption takes place even when both the hydroxylated alumina and the protozoa carry the same charge. The experimental work in question is

described in the specification of the present application at page 14, line 28 to page 15, line 10.

16. The alumina described in the Latimer patent does not have the properties of the hydroxylated alumina of the present invention. In this regard, the Latimer patent teaches the use of unhydroxylated alumina which we have shown experimentally to be ineffective in adsorbing protozoa oocysts.

The undersigned declarant declares that all the statements made herein of her own knowledge are true and that all statements made as information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both under the United States Code and that such wilful false statements may jeopardise the validity of the application or patent issuing thereon.

Dated this 27th March 2002

M. K

MARILYN KARAMAN

Applicant: The Australian National University and Australian Water Technologies Pty Ltd
Serial Number: 09/646347
Filed: 18 March 1999
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ANNEX A

The attached is Annex A referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. Ic

MARILYN KARAMAN

Curriculum Vitae

NAME: **Marilyn E. Karaman**

ADDRESS: 105 Bandjalong Cres., Aranda, ACT 2614

NATIONALITY: Australian

PROFESSION: Analytical and Physical (Surface) Chemist

EDUCATION/QUALIFICATIONS :

Chemistry Certificate , Sydney Technical College, (Completed in 1978)

Associate Diploma in Applied Science (Chemistry), Canberra College of Advanced Education (Completed in 1984).

Degree of Bachelor of Applied Science (Chemistry), Canberra College of Advanced Education (Completed in 1985).

Graduate Diploma in Science Pass with Merit (Chemistry), The Australian National University (Completed in 1991).

Graduate Teaching Program Certificate, The Australian National University, (Completed in 1995)

Degree of Doctor of Philosophy (Chemistry), The Australian National University (Awarded September 2000).

SUMMARY OF PhD EXAMINERS COMMENTS

Thesis entitled: "Experimental and Theoretical Studies on Surfactant Wetting and Surface Forces".

Examiners : Prof. Jim Quirk (University of Western Australia), Prof. Tom Healy (Melbourne University) and Dr Tommy Nylander (Sweden).

Examiner (1)

Stated that each chapter in the thesis was "cutting edge" in the designated topic, "self contained". "The thesis is truly a substantial contribution". "The candidate has demonstrated that she is a versatile and accomplished laboratory chemist" "the most noteworthy features of this thesis is the variety of experimental skills and techniques she has used". The various chapters were described as having "excellent and comprehensive set of experiments", "quite superb experiments with all the care demanded", "fascinating results", "the thesis taken as a whole is a tour de force".

"In terms of presentation the candidate writes with clarity and economy of style which to me indicates someone thoroughly conversant with the subjects treated and with their theoretical background and connotations". "The thesis is so well illustrated that many book authors could learn from the candidates approach".

"A substantial part of the research has been already been published in first class journals".

"Some Universities have a category cum laude and it is my opinion that Ms Karaman's thesis resides comfortably within this category. Accordingly I recommend that she should be favourably considered for a University Prize".

Examiner (2)

"It was a great pleasure to read this thesis as it covers such a broad spectrum of problems in surface and colloid science. Many of the experiments presented are really clever. The PhD candidate has used a number of techniques that by themselves are quite tricky yet she has obtained data of very good quality. The amount of work put in this thesis is impressive and more than what is usually expected for a PhD. The thesis also arises new, relevant and interesting questions, which I'm sure, will be topics for PhD theses in the future".

The various chapters were described as "impressive", "new" and "interesting" experiments "very clever and very well carried out".

Examiner (3)

"This thesis is the best produced one I have ever read : I congratulate the author" "Almost all of this thesis is a delight to read and study".

The various chapters have been described as "interesting, useful and worthwhile work" "thought provoking", "concise", "tantalising stuff" containing "some very elegant experimental work".

SUMMARY OF RELEVANT WORK EXPERIENCE:

Over the last 25 years I have gained extensive experience in laboratory practice and research in both analytical and physical chemistry. During my undergraduate studies I completed a major in Chemistry with electives in Medical Technology units. I have a strong background in Atomic Force Microscopy, TEM, SEM, organic synthesis and surface chemistry (both as a RA II and as a graduate student). I have also gained valuable experience in microbiological techniques, MLC, radioimmunoassays, radiolabelling (organic synthesis) and a feel for medical research in general, because of my involvement in immunosuppression research in the John Curtin School of Medical Research (JCSMR) at the ANU.

In addition, I have been involved in membrane design and production of a new generation of *filtration (ultra and desalination) membranes* and the design of a novel fully automated *instrument for the determination of the Minimum Film Formation Temperature of Latex.* I have recently been carrying out research involving the *removal of Cryptosporidium from water.* It should be noted that ***all three projects*** have resulted in the lodgement of patent applications naming me as an ***inventor.***

July 1974 - December 1975: Marrickville Holdings (ETA Division), *Trainee Chemist*, Analysing a wide range of food products, raw materials and in process foods.

December1975 - July 1977: A.P.D. Snack Foods, *Quality Control Analyst*, Analysing a wide range of food products,raw materials and in process foods.

July 1977 - September 1978: Soul Pattinson Laboratories, *Laboratory Analyst (Quality Assurance)*, Responsible for quality assurance analysis on a wide range of pharmaceutical products, raw materials and the in-process goods.

September 1978 - April 1980: Helena Rubinstein, *Quality Assurance Chemist*, Performing similar duties as above for a wide range of cosmetic products involving both chemical and instumental analysis as well as colour matching.

July 1980 - September 1985: Department of Chemistry, ANU, *Technical Officer*, I played a supporting role in the teaching laboratories involving the preparation of undergraduate starting materials and lecture demonstrations, preparation and standardisation of solutions required for practical classes. Recovery of precious metals. Demonstrating techniques involved in undergraduate radiochemistry experiments. Operating the AAS and 360M NMR as a service for undergraduate classes.

September 1985 - July 1997: Department of Applied Maths, Research School of Physical Sciences and Engineering, ANU, *Research Assistant Grade II*, Involving varied interdisciplinary research which in the past has involved immunology (radioimmuno -assays) - MLC, radiolabelling, organic synthesis, freeze fracture/freeze etch, TEM, SEM, membrane design using polymerisable microemulsions, imaging AFM, *Colloid Probe AFM* measuring forces in a variety of systems including between cast latex films in the presence of various additives.

August 1997 - November 1997: Department of Chemistry, The Faculties, ANU, *Research Assistant Grade II*, Measuring forces in a variety of systems using LLIFE (light lever instrument for force evaluation). LLIFE is an in-house built AFM dedictated solely for surface force measurements.

November 1997- April 1998: Australian Water Technologies - Ensight (West Ryde, Sydney), *Consultant*, Involved in interdisciplinary research, using surface chemistry techniques such as microelectrophoresis, FESEM and Atomic Force Microscopy to study the pathogenic protozoa *Cryptosporidium*.

May 1998- April 2000: Australian Water Technologies-Ensight, *Environmental Scientist*, Involved in environmental pathogens (e.g *Cryptosporidium*) and water treatment research. Standard surface chemistry techniques and AFM were used to study the flocculation process for a variety of water treatment chemicals and their interaction with environmental pathogens such as *Cryptosporidium parvuum*.

April 1998- April 2000: *Visiting Fellow* in the Department of Chemistry, The Faculties, Australian National University.

April 2000-Present: Department of Chemistry, The Faculties, ANU, Research Associate, measuring forces between fluorocarbon coated surfaces to elucidate the nature of the *Hydrophobic Interaction*.

Grants

ARC SPIRT Grant with AWT-Ensight (NSW) for 'Surface characterisation of *Cryptosporidium* oocysts for the development of novel filtration systems for commercial applications' ($140,000).

REFERENCES ARE AVAILABLE FROM:

(1) Professor Richard Pashley (Chair of The Board of The Faculties), The Australian National University, Email: richard.pashley@anu.edu.au.
Ph: 61-02 6249 2631.

(2) Professor Barry Ninham (Head of Department), Dept. of Applied Maths, Research School of Physical Sciences and Engineering, The Australian National University, Email: barry.ninham@anu.edu.au., Ph: 61-02 62492470.

(3) Professor J.P.Quirk, School of Agriculture, University of Western Australia, Nedlands WA 6009, Ph: 61- 08-93802769, Fax: 61-08-93801050.

(4) Professor Tom Healy (Head of Department), School of Chemistry, University of Melbourne, E-mail: t.healy@chemistry.unimelb.edu.au., Ph: 61-3-93446481.
fax: 61-03-93446233

REFEREED INTERNATIONAL JOURNAL ARTICLES AND PATENTS

(1) M.E.Karaman, L.Meagher and R.M.Pashley. Surface Chemistry of Emulsion Polymerisation, *Langmuir*, **9**, 1220-1227 (1993).

(2) M.E.Karaman, B.W.Ninham and R.M.Pashley. Some Aqueous Solution and Surface Properties of Dialkyl Sulfosuccinate Surfactants, *The Journal of Physical Chemistry*, **98**, 11512-11518 (1994).

(3) M.E.Karaman, R.M.Pashley and N.K.Bolonkin. Study of the Surface and Biological Activity of a Trivalent Cage Surfactant., *Langmuir*, **11**, 2872-2880 (1995).

(4) R.M. Pashley, B.W. Ninham, S.T. Hyde, M.E. Karaman and R.A. Morris 'Formation of porous materials.' US Patent No 5,529,690, issued 25 June 1996.

(5) M.E.Karaman, B.W.Ninham and R.M.Pashley. Effects of Dissolved Gas on Emulsions, Emulsion Polymerisation, and Surfactant Aggregation, *The Journal of Physical Chemistry* , **100**, 15503-15507 (1996).

(6) R.M.Pashley, M.E.Karaman, B.W.Ninham., Method and Apparatus for the Measurement of Film Formation Temperature of a Latex., *Provisional Patent Submitted* (February 1997).

(7) M.E.Karaman, R.M.Pashley, T.D.Waite, S.J.Hatch and H.Bustamante. A Comparison of the Interaction Forces Between Model Alumina Surfaces and Their

Colloidal Properties, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **129-130**, 239-255 (1997).

(8) V.Yaminsky, B.W.Ninham and M.E.Karaman. Dewetting of Mica Induced by Simple Organic ions Kinetic and Thermodynamic Study., *Langmuir*, **13**, 22, 5979-5990 (1997).

(9) M.E.Karaman, R.M.Pashley, H. Bustamante and S.R.Shanker., Method of Water Purification., *PCT International* (Publication date: September 1999).

(10) R.M.Pashley, M.E.Karaman, V.S.J Craig and M.M.Kohonen. Use of the Light-Lever Technique for the Measurement of Colloidal Forces., *Colloids and Surfaces A: Physicochemical and Engineering Aspects* , **144**, 1-3, 1-8 (1998)

(11) M.E.Karaman, R.M.Pashley, H.Bustamante and S.R.Shanker. Micro-electrophoresis of *Cryptosporidium* parvum Oocysts in Aqueous Solutions of Inorganic and Surfactant Cations., *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **146** n1/3, 217-226 (1999).

(12) R.M.Pashley and M.E.Karaman. The Role of the Meniscus in the Drying of Latex films, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, - In Press.

(13) M.M.Kohonen, M.E.Karaman and R.M.Pashley. Electrical Double Layer Forces in the Presence of Multivalent Electrolytes, *Langmuir* **16(13)**: 5749-5753, 2000.

(14) S.R.Shanker, M.E.Karaman, H.Bustamante and R.M.Pashley. Surface Characteristics of *Cryptosporidium* Oocysts: Implications for Pilot Plant Studies, *Journal of Environmental Microbiology* - Submitted.

(15) H.Bustamante, M.E.Karaman, S.R.Shanker and R.M.Pashley. Effect of Coagulants and Polyelectrolytes on the Surface Properties of *Cryptosporidium, Water Research*- In Press.

(16) M.E.Karaman, D.A.Antelmi and R.M.Pashley. Hydrocarbon and Fluorocarbon Carboxylic acid Adsorption onto Alumina Substrates, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, - In press.

(17)M. E. Karaman, R. M. Pashley, S.R. Shanker, and H. Bustamante
'Destruction of *Cryptosporidium* Oocysts by Adsorption onto Active Solids'
International PCT Application (Submitted March 2001).

REFEREED CONFERENCE PAPERS

S.R.Shanker, H.Bustamante,M.E.Karaman and R.M.Pashley. Relevance of the Surface Chemistry of Cryptosporidium Oocysts to Water Treatment Plants. Proceedings of the Xth World Water Congress, Melbourne Australia, February 2000

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ANNEX B

The attached is Annex B referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. K

MARILYN KARAMAN

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ANNEX C

The attached is Annex C referred to in the Declaration made by Marilyn Karaman on 27/3/02 2002

M. K

MARILYN KARAMAN

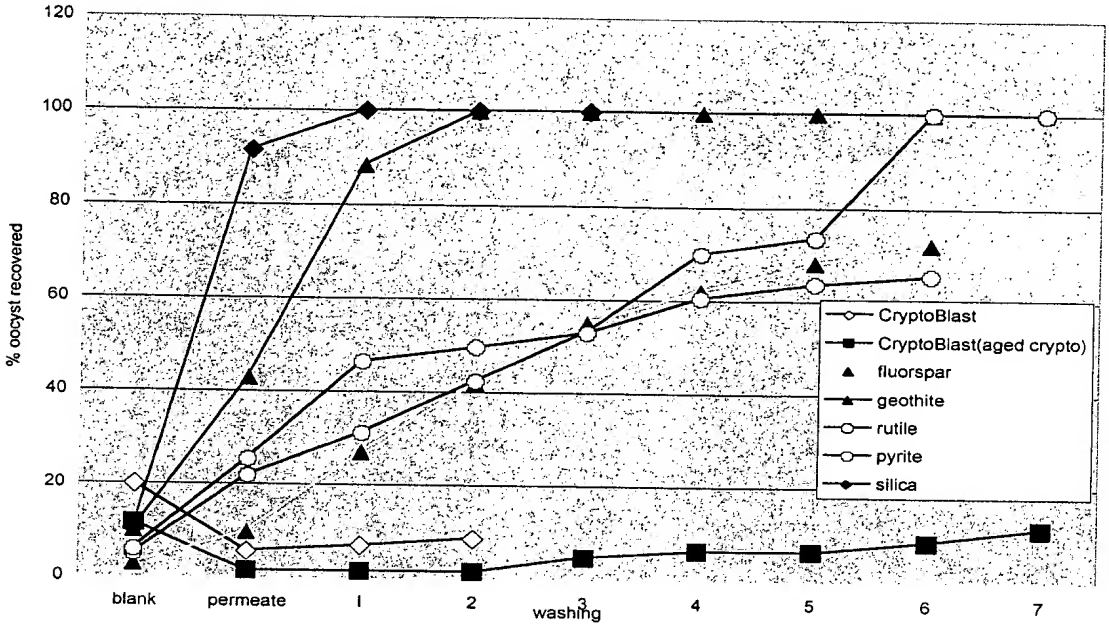

Figure 1. Comparison between hydroxylated alumina (CryptoBlast™) and a wide range of other inorganic solids on oocysts elution from column with water.

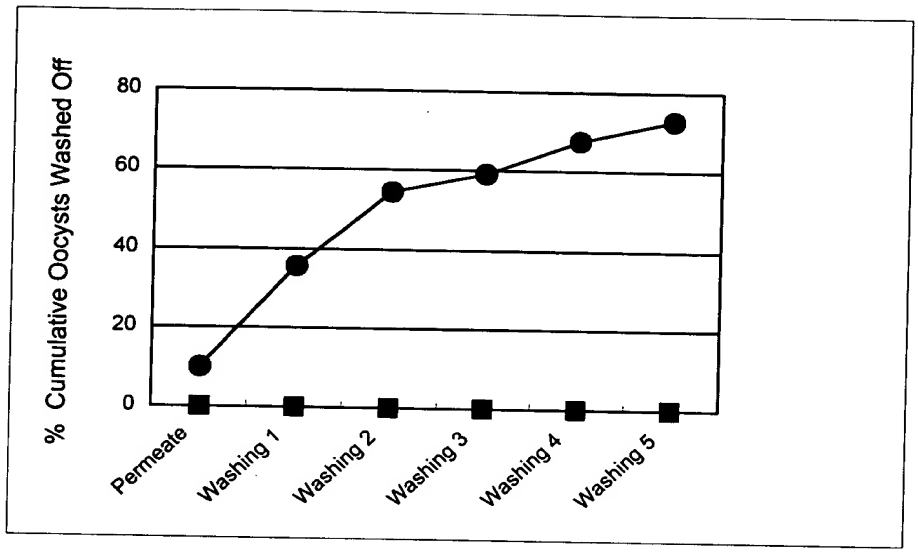

Figure 2. *Cryptosporidium* on unhydroxylated alumina (n) are readily removed by washing with water as opposed to *Cryptosporidium* on hydroxylated alumina (g) onto which the oocysts are irreversibly adsorbed